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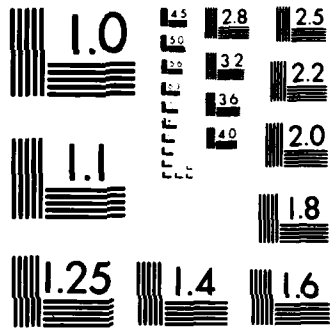

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Final Report

A. Herz

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A broad range of research activities were spanned in this annual meeting of the International Narcotic Research Conference. These included an emphasis on biochemical and pharmacological studies, as well as reports dealing with the physiological or pathophysiological functions of endogenous opioid systems. The relevance of these areas of basic science to the clinical domain was also emphasized in a few reports. The interdisciplinary nature of these meetings again resulted in a synthesis of new approaches to biomedical research in the area of endogenous opioid systems.

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Report on 1983 INTERNATIONAL NARCOTICS RESEARCH CONFERENCE Meeting

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The 1983 INRC was held in Garmisch-Partenkirchen, West Germany, a Bavarian resort town about 1 hour by car or train from Munich. The meeting was hosted by a local Organizing Committee headed by Prof. Albert Herz, Max Planck Institute for Psychiatry, Munich. The meeting began with a morning session on Monday June 27 and ended at noon on Friday July 1. There were morning and evening sessions as well as poster sessions from 4:30-6:30 PM.

This year for the first time there were four invited speakers who were outstanding scientists in other fields, with expertise of particular interest to the participants. These lectures were held from 12:15-1:15 every day except Wednesday and Friday. In spite of the crowded schedule attendance was excellent. The meeting was attended by 338 scientists from 23 countries. The United States was represented by more than one hundred participants.

As usual, the INRC spanned the broad range of research activities in the opioid and related fields. It was heaviest in biochemical and pharmacological reports but there were also physiological, behavioral and a small number of clinical papers.

I shall briefly summarize what I view to be some highlights of the results presented this year. I shall end with a brief evaluation, a word about its significance, reasons for meeting dates and places and future plans.

Scientific Highlights

Opioid Receptors

One of the biochemical areas in which progress is beginning to accelerate is in the solubilization and partial purification of opioid binding sites.

Dr. R.S. Zukin and collaborators (NY,NY) reported partial purification of opioid binding sites solubilized from rat brain with the detergent CHAPS. Partial purification was achieved on an affinity column in which a mercurial bromide derivative of thebaine synthesized in Dr. S. Archer's laboratory, was attached to agarose beads containing SH groups on their side chains. The bmax of the purified preparation was 3000 fmol/mg protein, representing an approximately 30 fold purification over receptor specific activity in the membranes. Attempts to purify opioid receptors solubilized from rat brain by glycodeoxycholate on an affinity column bearing $Dala^2Met^5$ enkephalin were reported by Nagai et al. (Tokyo, Japan). However, these trials are only in

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early stages. Demoliou-Mason and Barnard (London, GB) reported that they were able to solubilize rat brain opioid binding sites with digitonin in the absence of high concentrations of NaCl. The requirement for high salt, first reported by Howells et al., was abolished by changing the extraction buffer from Tris to TES.

Bidlack et al., (Rochester, NY) reported that they have succeeded in producing at least two monoclonal antibodies that seem to inhibit specific binding of opiates. Their evidence suggests, but does not yet prove, that these antibodies are directed against the receptors. Purification and characterization of the antibodies is in progress.

There was a large number of papers dealing with the study and characterization of opioid receptor types. Only a few results can be mentioned in this brief summary. Dr. Itzhak et al., (New York,NY) did sucrose gradient centrifugation of opioid binding sites solubilized from rat brain with digitonin. He found that he was able to separate the binding sites for mu and delta ligands from those for kappa ligands. The molecular weight of the kappa sites was about 400,000 while the mu and delta sites seemed to have a molecular weight of ca. 700,000. Another very interesting paper came from Jauzac et al. (Toulouse, France). They solubilized opioid receptors from rabbit cerebellum, a tissue they had previously shown to contain mainly mu binding sites. The sites were prelabeled with either the agonist ^{3}H-etorphine or the antagonist ^{3}H-diprenorphine. Sucrose density gradient centrifugation gave two separate radioactive peaks, i.e., the main peak labeled with etorphine sedimented faster than the major peak prelabeled with diprenorphine. The authors feel that these results constitute the first direct evidence for the existence of physically distinct agonist and antagonist forms of the mu opioid receptor.

The use of covalent affinity labeling agents to purify and identify opioid receptors and their subunits is also beginning to meet with some success. The best example of such a study is the paper of Simmonds et al. (Bethesda, MD). These workers have used ^{3}H-fentanylisocyanate to label covalently the delta receptors in rat brain and NG108-15 cells. A single labeled protein of molecular weight 58000 was identified by SDS-polyacrilamide gel electrophoresis. This is a glycoprotein which is not labeled in the presence of excess opioid receptor ligands and is, therefore, thought to be a subunit of the receptor.

B. Roques et al. (Paris, France) reported the synthesis of quite selective photoaffinity labeling agents for mu and delta opioid binding sites. The para-azido-phenylalanine derivatives of Tyr-D-Ala-Gly-(NMe)-Phe-Gly-ol (DAGO) and of Tyr-D-Thr-Gly-Phe-Leu-Thr (DTLET) were found to inactivate mu and delta sites sselectively after irradiation at 254 nm.

There were both oral paper and poster sessions on the topic "tolerance and dependence" from which I will pick only a few reports.

Parenti et al. (Milan, Italy) presented quite convincing evidence that opiates stimulate a high affinity GTPase in rat striatal membranes, a result that had previously been reported only for NG-108-15 cells in culture. In striatal membranes prepared from morphine-dependent rats there was a significant decrease in GTPase activity. This supports the notion that changes in coupling between adenylate cyclase and opioid receptors may play a role in the development of tolerance and dependence.

Using βCNA and βFNA administered via spinal catheters, Takemori et al. (Minneapolis, MN) provided evidence for a role of mu opioid receptors in the development of tolerance and dependence to systemically administered morphine.

Smith et al., (Ann Arbor, MI) reported that chronic treatment with opiates decreased the number of alpha2 adrenoreceptors (bmax) without changing the affinity of clonidine binding. In this respect there was little difference between mu and kappa agonists.

The fascinating topic of receptor down-regulation and internalization was discussed under this heading. For many years it was thought that opioid receptors were difficult to regulate and down-regulation was only demonstrated in the last one or two years in several laboratories. Chang et al. (Triangle Park, NC), who previously reported on down-regulation in NG-108-15 cells, now reported that they can observe similar regulation in hippocampal slices. Prolonged incubation with delta agonists was effective, whereas incubation with mu agonists was not. Lenoir et al. (Rehovot, Israel) used aggregating fetal rat brain cells in culture to show that delta receptors are not the only ones that can be down-regulated, but evidently this can also be observed for mu and kappa receptors.

Down-regulation has in the case of other receptor systems been found to be due to internalization and metabolism of the receptors. Law et al., (San Francisco, CA) presented some evidence that suggests a similar mechanism for opioid receptors at least in NG-108-15 cells. When cells were treated with chloroquine which interferes with lyzosomal enzyme function by changing the pH inside the lyzosomes, these workers found a time-dependent increase in cell-associated bound ^{3}H-DADL. The radioactivity was no longer sensitive to trypsin nor exchangeable by excess diprenorphine. Cell fractionation suggested that the bound receptors were associated with the lyzosomal fraction.

Opioid Peptides

There have also been some very significant advances in our knowledge of the opioid peptides. One ingenious approach was to look at the prohormones and determine sites of possible processing (single or double basic amino acids).

By preparing antibodies to a sequence that might be generated from pro-enkephalin by a single arginine cleavage Weber et al. (Stanford, CA) were able to show that large quantities of such a peptide exist in the brain. The octapeptide exists in an amidated form. It has the structure Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val NH_2 and has been named metorphamide.

Using a similar approach Nakao et al. (Kyoto, Japan) raised the question whether a 29 amino acid peptide present in prodynorphin might exist. This polypeptide, which contains dynorphin B (rimorphin) at its N-terminal, was indeed found to exist in porcine neurointermediate pituitary and has been called leumorphin by the authors.

There was a considerable number of papers on coexistence and corelease of opioid peptides with other neuropeptides and neurotransmitters. Thus Rossier's group (Gif-sur-Yvette, France) characterized the nature of the enkephalin-like material co-released from the adrenal medulla with catecholamines. When stimulation was relatively gentle, i.e. when the splanchnic nerve was stimulated electrically, the material released was fully processed enkephalin. When, however, the stimulation was quite strong and non-physiological, i.e. 50 mM KCl, the material released was largely in the form of high molecular weight enkephalin precursors.

An important area which has been obscure up till now is the nature of the enzymes that process the precursors to form the final opioid peptides. There were several reports on progress in this area. Fricker and Snyder (Baltimore, MD) reported the purification of both a membrane-bound and a soluble carboxypeptidase which has properties consistent with a function as an enkephalin synthesizing enzyme. The "convertase" seems to be a Co^{++} stimulated enzyme whose distribution in the brain parallels very closely that of the enkephalins. Hook and Eiden (Bethesda, MD) reported the presence of a trypsin-like activity in the chromaffin granules of the adrenal gland. They had previously reported the presence of a carboxypeptidase B-like activity in these granules. They feel that these enzymes may represent the enzymes involved in the processing of proenkephalin to enkephalin.

Physiological, Pharmacological and Behavioral Reports

A number of papers addressed the distinctive behavioral and physiological actions of kappa agonists including the dynorphins. Hayes et al. (Ware, G.B.) confirmed their previous findings that kappa agonists are analgesically potent against mechanical noxia but relatively ineffective against thermal noxia. This profile was shown to extend to dynorphin 1-17,1-13, and 1-8 which were injected either intracerebroventricularly or intrathecally. Calthrop and Hill (Cambridge, G.B.),however, were unable to demonstrate a correlate of this profile on the level of single cell nociceptive responses. The excitatory responses of neurons in the trigeminal sensory nucleus to thermal and mechanical pain stimuli were similarly inhibited by a range of kappa agonists.

Immunchistochemical and electrophysiological studies supported the existence of unique spinal analgesic mechanisms in which the dynorphins are active.

Przewlocki, et al. (Munich, W. Germany) reported a preferential distribution of spinal dynorphin immunoreactive material dorsally in the lumbo-sacral cord. A particularly high density was found in the substantia gelatinosa which is considered the locus of Melzack and Wall's "pain-gating" mechanism. Moreover, Werz and Macdonald (Ann Arbor, MI) demonstrated the existence of primary somatosensory neurons in the dorsal root ganglia in which dynorphin, but not morphiceptin or leu-enkephalin, decreases the calcium-dependent action potential duration. Evidence for a supraspinal kappa analgesic mechanism was also presented. Satoh et al. (Kyoto, Japan) reported that while both morphine and EKC produce analgesia when injected into the nucleus reticularis paragigantocellularis, only morphine analgesia is blocked by a low systemic dose of naloxone, confirming a previous report by Carr and Simon.

A complication underscored by several papers is that kappa agonists apparently produce some of their effects by interacting with a non-opioid receptor. For example, while dynorphin and dynorphin 1-8 produce analgesia when injected interthecally, only the effect of dynorpin 1-8 was found by Przewlocki, et al. (Munich, W. Germany) to be naloxone reversible. Moreover, Des Tyr dynorphin, which does not interact with opioid receptors, produces a similar analgesia. Faden and Jacobs (Washington, DC) demonstrated that a unique behavioral effect of intrathecally injected dynorphin is the production of paralysis. Paralysis is not, however, naloxone reversible and is also produced by des-tyrosine dynorphin. The low affinity binding site for kappa agonists and dynorphins described by Attali, et al. (Toulouse, France), which does not bind the non-kappa opiates and opioids, may be mediating some of these effects.

A number of interesting findings were reported regarding the regulation of feeding behavior by endogenous opioid activity. Supporting the involvement of central rather than peripheral opioid activity in the acute facilitation of feeding, Carr and Simon (NY,NY) reported that electrical brain stimulation threshold for eliciting eating is elevated by naloxone but not by its quaternary analogue. Further, when the peripheral component of morphine's net effect on feeding is blocked by co-administration of quaternary naloxone, thresholds for eliciting feeding are reduced. Specific brain regions and peptides involved in the mediation of feeding were indicated as well. Schulz and Wilhelm (Munich, W. Germany) showed that microinjection of anti-α-neoendorphin antibodies into the ventromedial hypothalamus substancially reduced feeding in food-deprived rats. The reduction was significantly greater than that produced by antibodies to beta-endorphin or dynorpin 1-13. The involvement of extra-hypothalamic loci was also suggested by the work of Mucha and Iversen (Cambridge, G.B.). They reported that opioid agonists injected into the nucleus accumbens substantially increase food intake. Moreover, the stereospecificity and naloxone-reversiblity of this effect were verified. The specific function served by central opioid activity in promoting eating was suggested by the results of Carr and Simon (NY,NY). By studying the effects of various hunger manipulations on brain

stimulation-induced eating and reinforcement in naloxone treated animals, these workers concluded that the anorectic effect of naloxone is not due to a direct suppression of appetite nor to increased gain in a satiety mechanism. Rather, naloxone appears to block the potentiating effect of hunger on a reward process that maintains feeding behavior.

Evaluation and Comments

INRC began with an informal meeting as satellite to the 1969 International Congress of Pharmacology, Basel, Switzerland. This little meeting was organized by Professors Kosterlitz and Collier. With one exception, a meeting has been held every year since. Under the leadership of Prof. A. Goldstein, the organization became more formally organized and received the name International Narcotic Research Club, later changed to Conference because of the frivolous connotations associated with the term club. The elected "Secretaries" led the INRC for 4 years each. Prof. Goldstein was followed by Prof. Sydney Archer, who was followed by E.J. Simon. The next Secretary will be Prof. E.L. Way.

There is general agreement that INRC has become the most important and authoritative meeting in the area of basic neuroscience research on exogenous and endogenous opioids. Its quality and prestige have been excellent and this year was no exception. 270 papers were presented and many of them were excellent. One of the reasons, in my view, for the high quality and prestige of the meeting is the fact that most of the directors of the top laboratories in the field attend the meetings themselves regularly. There were also many young investigators ranging down to post-doctoral trainees and some graduate students. This bodes well for the future of opioid research.

The invited speakers with expertise in other fields were received enthusiastically. All four lectures were superb, but those by Prof. Changeux and Prof. Lundberg were especially popular.

The Proceedings of this Conference will be published as a supplementary volume to Life Sciences. Participants were asked to submit only those papers that were not already published or in process of being published elsewhere. They exhibited extraordinary restraint since only 155 of the 230 papers presented were submitted for publication. We will furnish NIDA 6 copies of the Proceedings.

A word should be said about the date and location of the meeting. It is usually held some time during the summer in order to permit teaching scientists to attend. Since we are an international organization with a large American component the rule of thumb has been to meet in the USA two out of three years. This has been adhered to quite closely except for this year. This year INRC should have met in the USA. However, there were two reasons why we ended up meeting in Europe. 1) the very active group in Munich under the leadership of Prof. Albert Herz had been trying unsuccessfully to host the INRC in or near

Munich for four or five years, and 2) since the 1980 meeting of INRC was in Kyoto, Japan as a satellite to the Pharmacology Congress in Tokyo, there had been no meeting of INRC in Europe since 1977.

In future INRC will, when possible, meet as a satellite to IUPHAR every 3 years and in the USA, when possible as satellite to CPDD, during the other years. Next year the meeting will be held at Churchill College, Cambridge, U.K. July 23-27, as a satellite to IUPHAR which will be held in London July 29-Aug 3, 1984.

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